

Effect of Host Instar on Measuring Parasitism of *Lygus* spp. (Hemiptera: Miridae) Nymphs by *Peristenus* spp. (Hymenoptera: Braconidae)

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ABSTRACT The accurate measurement of insect mortality by parasites is critical in biological control research, both in baseline studies to determine the absence or inadequacy of native parasites and in subsequent efforts to measure the effectiveness of introduced endoparasitic species. Although rearing has been most frequently used to measure parasitism, dissection has been shown to be more accurate in several cases. Selection of the host instar, whether for rearing or dissection, was also found to be important in this study. In two species [*Lygus lineolaris* (Palisot) and *L. hesperus* Knight], parasitism by *Peristenus digoneutis* Loan and *P. howardi* Shaw, respectively, was highest in instars 3 and 4. Parasitism was underestimated in instars 1 and 2 (because of reduced exposure time) and in instar 5 (because of parasites killing the hosts in instar 4).

KEY WORDS *Lygus hesperus*, *Lygus lineolaris*, *Peristenus digoneutis* and *howardi*, measuring parasitism

The accurate measurement of insect mortality by parasites is critical in biological control research, both in baseline studies to determine the absence or inadequacy of native parasites and in subsequent efforts to measure the effectiveness of introduced parasite species.

The most frequently used method to measure parasitism by endoparasites has been rearing the host (Day 1994, Perez-Mendoza et al. 1999, Brewer et al. 2001, Simmons et al. 2002, Alma et al. 2005, and Bahlai et al. 2006). Numerous additional references are in Day (1994). The latter paper showed that the rearing procedure can significantly underestimate parasitism, because parasitized individuals are likely more stressed, so will often have a higher mortality rate than unparasitized individuals. Their premature deaths will also kill their parasites, reducing the apparent parasitism rate. In such cases, Day (1994) recommended that two methods be used: rearing, to obtain the adult parasites usually required to easily (or more economically) identify the species of parasite; and dissections, to more accurately measure the percentage parasitized.

If the dissection method is to measure parasitism of immature insects (nymphs or larvae), consideration also should be given to the instar that will be dissected. In theory, because early instars are exposed to possible attack by parasites for a shorter total period of time than older instars, parasitism rates should be higher in the later instars. However, it is also possible that par-

asite larvae that result from eggs laid in early host instars may complete development before the host's final instar is reached, eliminating parasitized hosts, so parasitism may appear to decrease in the late instars.

These two hypotheses were tested with two species of mirids: the tarnished plant bug, *Lygus lineolaris* (Palisot) collected in northwestern New Jersey and the western tarnished plant bug, *Lygus hesperus* Knight, collected in southwestern Idaho. Nymphs of both species are attacked in alfalfa fields by the braconid parasites *Peristenus digoneutis* Loan (an introduced species) in the northeastern United States (Day et al. 1990, 2003, Day 2005) and *P. howardi* Shaw (a native species) in the northwestern United States (Day et al. 1999, Seymour et al. 2005). Both parasites oviposit in *Lygus* nymphs (Day et al. 1990, 1999).

Materials and Methods

Nymphs of the two mirid species were collected by sweep net at weekly intervals from alfalfa fields at two locations: *L. lineolaris* near Blairstown, NJ (Day 1996) and *L. hesperus* at Parma, ID (Seymour et al. 2005). The mirids were placed in an insulated cooler with a frozen coolant for transport to Newark, DE, by car (*L. lineolaris*) or by overnight courier (*L. hesperus*). At Newark, each sample was divided into two aliquots of 30–60 individuals. One aliquot was frozen at –18°C (0°F) for later dissection (at 7–20 ×), and the other aliquot was placed in a rearing cage with alfalfa “bouquets” as food in an environmental chamber (22–24°C,

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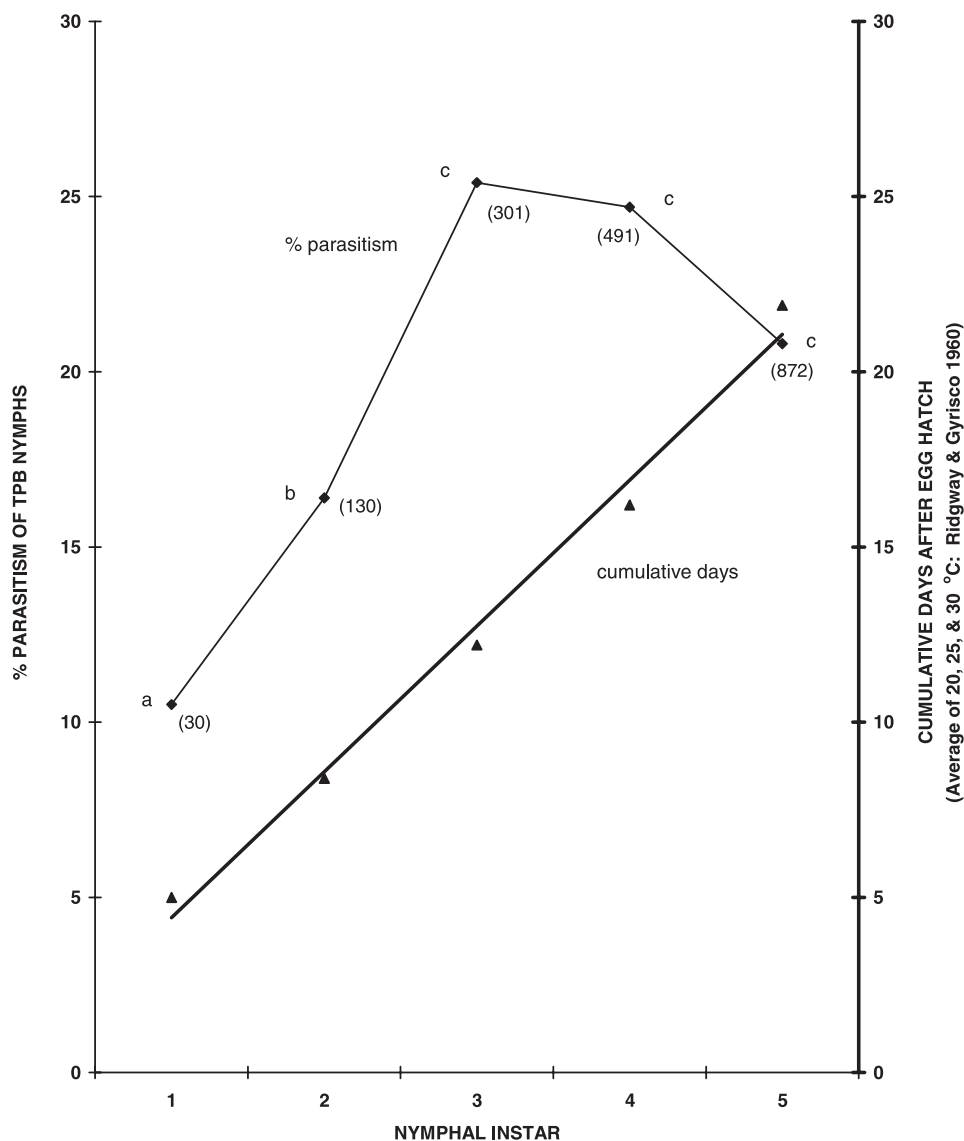


Fig. 1. Parasitism of tarnished plant bug (*L. lineolaris*) nymphs by *P. digoneutis* versus host instar and time. Blairstown, NJ, 1987–1992 and 1998–2003 data. Different letters (Duncan's test) are significantly different at 1% (ANOVA, $df = 4,44$; $F = 5.15$; $SEM = 1.74$). Numbers in parentheses are the number of nymphs of each instar that was dissected.

60–70% RH, 16:8 PP) for emergence of parasite adults to identify the parasite species.

Data from 1,824 dissected nymphs of *L. lineolaris* are included here. These were collected during two 6-yr time periods: 1987–1992 (1,149 nymphs), when tarnished plant bug populations had not yet been suppressed by the recently established *P. digoneutis* (and parasitism rates averaged 30%), and 1998–2003 (675 nymphs), when tarnished plant bug numbers had been markedly reduced by the introduced parasite, and average parasitism had fallen to 10%. The two samples increased the statistical significance by enlarging the sample size. Each year, there usually were two larger and one smaller generations of *L. lineolaris*

nymphs, occurring in June, July–August, and September, respectively.

Data for the 1,736 dissected nymphs of *L. hesperus* from Idaho were less variable, so results from only one 6-yr period (1998–2003) are included here. There were two major generations of nymphs in June and July.

All percentage data were converted to arcsines for the analyses of variance, and mean parasitism rates for each instar were compared using Duncan's multiple range test (Steele and Torrie 1980). Parasitism rates for instars 3 and 4 were compared with the parasitism of all instars by the χ^2 test.

Developmental time data for each of the five instars of *L. lineolaris* were obtained from Ridgway and

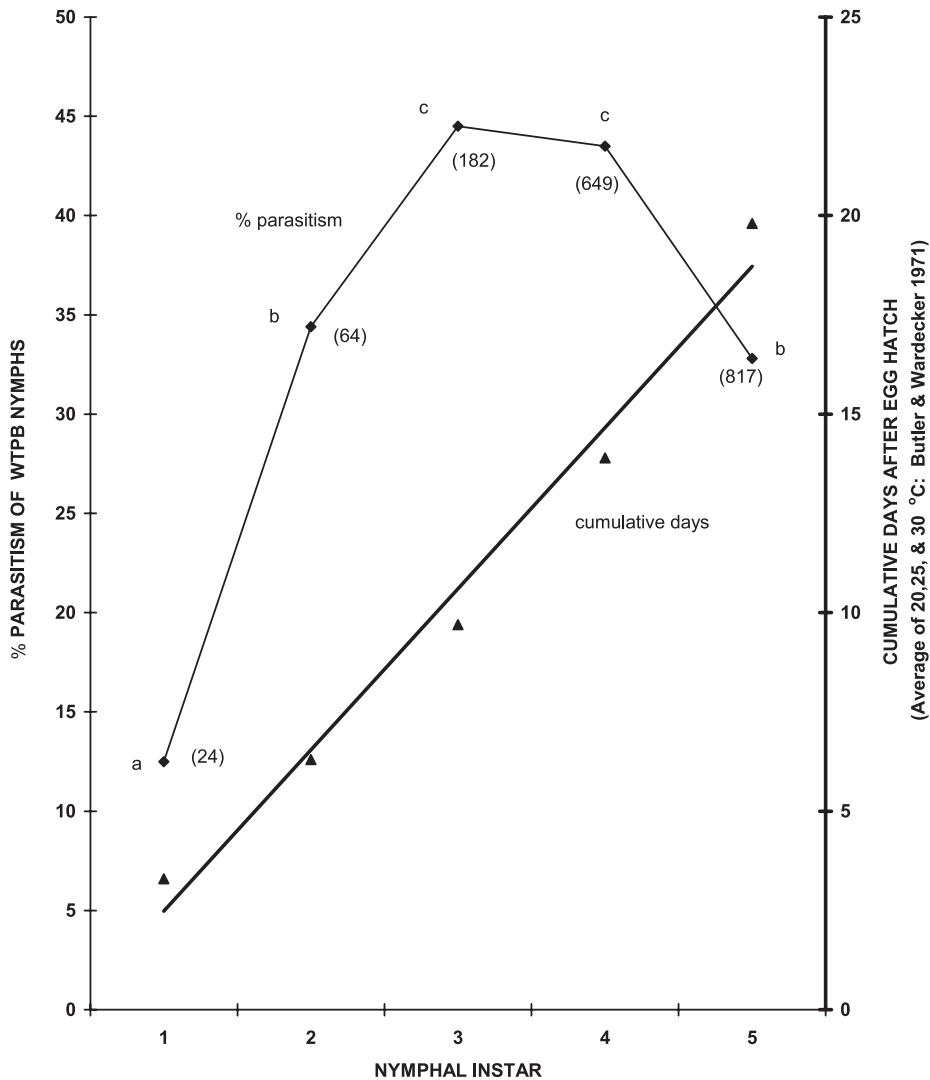


Fig. 2. Parasitism of western tarnished plant bug (*L. hesperus*) nymphs by *P. howardi* versus host instar and time. Parma, ID, 1998–2003. Different letters (Duncan's test) are significantly different at 1% (ANOVA, $df = 4,20$; $F = 18.8$; $SEM = 1.96$). Numbers in parentheses are the number of nymphs of each instar that was dissected.

Gyrisco (1960), and an average was calculated for three temperatures (20, 25, and 30°C). Similar averages were also calculated from *L. hesperus* data in Butler and Wardecker (1971) for the same three temperatures.

Results and Discussion

Development Time of *Lygus* spp. Nymphs. Nymphs of the two species developed at very similar rates, reaching the fifth instar in ≈ 20 d (Figs. 1 and 2), based on data in Butler and Wardecker (1971) and Ridgway and Gyrisco (1960).

Parasitism Species in the Two Areas. All parasites reared from Idaho were *P. howardi*. Most parasites reared from the New Jersey samples were *P. digoneutis* (74% in 1987–1992, and 89% in 1998–2003); the re-

maining species here were *P. pallipes* (Curtis) and *Leiophron uniformis* (Gahan).

Parasitism Rates. Maximum parasitism of both tarnished plant bug species was observed in the third and fourth instars (Figs. 1 and 2). This seemed to be caused by three characteristics: the longer total time that older nymphs have been exposed to parasite attack; the difficulty in detecting the very small and transparent *Peristenus* eggs, most of which are deposited into first and second instars (unpublished data); and parasite-induced mortality of some nymphs before they reach the fifth instar. The data suggest that parasites that begin development in early-instar nymphs mature (and kill their hosts) before the latter reach their fifth instar, causing an apparent decrease in parasitism in both *Lygus* spp. in fifth-instar nymphs (Figs.

Table 1. Comparison of parasitism rates of each instar of two species of *Lygus* nymphs and of instars 3 and 4 versus a random sample of all instars for both species

Instar	Percent parasitism by dissection		Both species
	<i>L. lineolaris</i>	<i>L. hesperus</i>	
1	10.5	12.5	
2	16.4	34.4	
3	25.4	44.5	
4	24.7	43.5	
5	20.8	32.8	
All instars ^a	22.0	37.8	29.6
Instars 3 + 4 ^a	24.9	43.7	34.5 ^b
Percent underestimation			14.2
Sample size	1,824	1,736	

^a These averages were weighted by sample size.

^b Significantly higher than the average of all instars ($\chi^2 = 0.005$, df = 1).

1 and 2). The dead fifth-instar nymphs cannot be collected from the field and later dissected or reared.

Selecting Instars to Measure Parasitism. The data for both *Lygus* spp. and their parasites (Figs. 1 and 2) showed that maximum parasitism occurs in instars 3 and 4. Because development of parasites in mirid nymphs takes >2 wk, over several host instars (Carignan et al. 1995), parasitism will gradually increase until the mature parasites kill the host, causing an apparent reduction in parasitism. Therefore, if random samples (including instars 1, 2, and 5) had been dissected, mortality by these *Peristenus* spp. would have been underestimated by 14% (Table 1). It should also be noted that significant immediate mortality of *Lygus* nymphs (Day 1994) and gypsy moth (*Lymantria dispar* L.; Lepidoptera: Lymantriidae) larvae (Fuester and Taylor 1993) caused by oviposition trauma has been observed and that adults of some parasite species kill hosts by feeding on them (DeBach 1943), so dissection of any instar is still likely to underestimate the total mortality caused by parasites.

The only insect disease detected in the dissections was a fungus, present in <1% of nymphs. This indicates that disease was not likely to be affecting parasite survival significantly by killing parasites or parasitized hosts. Predation in the field was also not likely to have influenced parasitism rates, because the reduced parasitism observed in fifth-instar nymphs could only occur if parasitized fifth-instar nymphs were preferentially attacked by predators.

In summary, the results of this study showed that dissections of random instars of immature mirids considerably underestimate mortality by parasites. This extends previous research that showed that the commonly used rearing method can significantly underestimate mortality by parasites compared with dissection. It is also possible that, if different parasite families or orders are present in an immature insect species, each may be most numerous in different host instars, so this also should be studied. It is hoped that researchers will extend similar evaluations to additional families and orders of insects to more accurately estimate the effectiveness of parasites.

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